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CHEMICAL PROFILE ANALYSIS AND ANTI-INFLAMMATORY ACTIVITY OF POLAR FRACTION FROM SOYBEANS (GLYCINE MAX)

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KEYWORDS: Glycine max soybean, polar components, HPLC fingerprint, anti-inflammation

INTRODUCTION

Presently, phytochemicals from soybeans have received more attention. They are used as nutraceutical ingredients due to their numerous health beneficial effects. Soy saponins, the main polar components, which exhibited in vitro antioxidation and anti-inflammation (1-4), as well as cytotoxicity to several cancer cell lines such as Hep-G2(5), Hela (6) and CaCo-2 (7-8) are among of interest. In this study, we investigated on chemical profile of soybean's polar fraction and its in vivo anti-inflammatory activity. HPLC-PDA-ELSD was employed for the chemical fingerprint analysis.

MATERIALS AND METHODS

Plant material: Soy beans were purchased from health food shop (Lemon green) in Bangkren District, Bangkok, in October 2011. They were pulverized into coarse powder (20 mesh), then exhaustedly soxhletted with hexane to give the defatted soy bean powder (DFP).

Extraction and fractionation: DFP 1.1 kg were macerated with 11 L of 70% ethanol for 72 h with occasional stirring, then filtered. After removal of ethanol under reduced pressure, 300 ml portion of aqueous solution equivalent to 360 g of DFP was extracted with 3x200 ml of n-butanol. The combined n-butanol solution was concentrated under reduced pressure to give 4.9 g of dried extract (BuFr).

HPLC analysis: BuFr was dissolved in methanol (1 mg/ml) and PTFE 0.45 µm filtered, then subjected to FIPLC analysis. Confirmation of components was done by comparison of Rt and absorbance spectral with reference standards.

Analyzing condition:
HPLC: Waters Pump600
Column: Nova-Pak C18 (150 mm x 3.9 mm i.d., particle size 4 µm)
Mobile phase: 0.1% trifluoroacetic acid in water (A) and acetonitrile (B), gradient elution: 100A to 62A/38B for 20 min, 62A/38B isocratic for 5 min, 62A/38B to 87A/13B for 25 min.
Flow rate: 0.8 ml/min
Inject volume: 10 µl
Detection: Photo diode array (PDA) at 205 nm and evaporative light scattering detector (ELSD) at probe temperature 50°C, a gain of 6.0 and nebulizer nitrogen gas of 3.3±0.1 bar.

Reference standards:
Soy isoflavones: daidzin, glycitin, genistin, daidzein, glycitein, genistein (Chromadex USA)
Soy saponins: soyasaponin I (Chromadex USA)

Anti-inflammatory Test (10)
Animals: Male Wistar rats were purchased from Laboratory Animal Centre, Mahidol University, Salaya, Nakornprathom, Thailand. The animals were housed in the animal facility of Thailand Institute of Scientific and Technological Research under standard conditions (25±2°C), 50-60% of humidity and 12 h/12 h light/dark cycles. The animals were kept under laboratory conditions for one week prior to the start of the experiments. Food and water were allowed ad libitum.

Carrageenan induced paw oedema: Rats weighing 80-100 g were divided in groups of six: vehicle control (distilled water), positive control (diclofenac 50 mg/kg), test sample I (BuFr 100 mg/kg) and test
sample II (BuFr 200 mg/kg). At the beginning of the experiment, initial paw volumes were determined using a water plethysmometer (Ugo Basil, Italy). Then, individual animal group orally received sample accordingly. One hour after sample administration, paw oedema was induced by injection of 0.1 ml of carrageenan (λ-carrageenan, type IV, Sigma) diluted in saline in the right hind foot pad. Paw volumes were determined at times 1, 2, and 3 h after oedema induction. The percentage of oedema inhibition was calculated with reference to vehicle control group.

**Analysis of Data**

The results are expressed as means ± S.E.M. Differences in mean values between groups were analyzed by a one-way analysis of variance (ANOVA).

**RESULTS**

**HPLC analysis**

HPLC chromatogram of BuFr detected under PDA at 205 nm (Fig. 1D) showed the presence of isoflavones daidzin, glycitin, genistin, daidzein, glycitein and genistein at Rt of 16.09, 16.90, 20.66, 29.70, 30.58 and 32.66, respectively as confirmed by Rt of standard isoflavones (Fig. 1A). Peak corresponding to soyasaponin I was observed at Rt 39.28 confirmed by ELSD (Fig. 1B, 1C). Among isoflavone constituents, genistein appeared to be the main component followed by genistin and daidzein, respectively.

**Anti-inflammatory activity**

BuFr at 100 and 200 mg/kg p.o. exhibited oedema inhibitory effect at time 2 and 3 h similar to that of diclofenac 50 mg/kg (Table 1). However, the effect seemed to be less potent and less lasting than diclofenac. Maximum effect of BuFr was observed at 2h while that of diclofenac appeared to be not less than 3 h. Interestingly, the anti oedema effect of BuFr was dose dependent manner at time 2 and 3 h.
Table 1. Oedema inhibitory effect of polar fraction, BuFr from soybeans tested by carrageenan induced paw oedema in rat.

<table>
<thead>
<tr>
<th>Samples</th>
<th>% Oedema</th>
<th>Oedema inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 h</td>
<td>2 h</td>
</tr>
<tr>
<td>Control</td>
<td>31.06±2.27</td>
<td>63.40±7.84</td>
</tr>
<tr>
<td>Diclofenac 50 mg/kg</td>
<td>23.44±1.93*</td>
<td>20.92±3.60*</td>
</tr>
<tr>
<td>BuFr 100 mg/kg</td>
<td>38.66±2.24*</td>
<td>22.25±3.73*</td>
</tr>
<tr>
<td>BuFr 200 mg/kg</td>
<td>25.68±3.07*</td>
<td>31.23±3.21*</td>
</tr>
</tbody>
</table>

n = 6, *significant from control, P < 0.05

% Swelling = \((T_0 - T_1) \times 100 / T_0\)

DISCUSSION

Chemical profile of polar fraction from defatted soybeans showed the presence of the representative phytochemicals including isoflavones and saponins. Seven components could be characterized using HPLC-PDA-ELSD including daidzin, glycitin, genistin, daidzein, glycitein, genistein, and soyasaponin I. Structurally, there have been no UV active chromophores in saponin molecule. Thus, PDA detector at 205 nm gave a low intensity peak of soyasaponin I while the saponin selective ELSD detector gave higher intensity peak. In considering only saponin components, ELSD is recommended.

**In vivo** anti-inflammatory activity was selected for our preliminary pharmacological assessment of BuFr since many pathological disorders are associated with inflammation. BuFr at 100-200 mg/kg exhibited a moderate anti-inflammatory activity compared to the standard anti-inflammatory drug, diclofenac at 50 mg/kg. The result is in accordance with its chemical profile and previous **in vitro** anti-inflammatory activity (1-2). So BuFr is interesting to be used as a natural nutraceutical ingredient for anti-inflammatory purpose.

CONCLUSION

\(n\)-Butanol fraction from the 70% aqueous ethanolic extract from defatted soybeans comprised both isoflavones and saponins detected by HPLC-PDA-ELSD. Seven components could be characterized from HPLC-PDA profile at 205 nm including daidzin, glycitin, genistin, daidzein, glycitein, genistein, and soyasaponin I. The fraction given orally at 100 and 200 mg/kg showed **in vivo** anti-inflammatory effect tested by carrageenan induced rat paw oedema.

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REFERENCES